RESEARCH PLAN PROPOSAL

COMPARATIVE PHYTOCHEMICAL STUDIES OF TWO VARIETIES OF *CYAMOPSIS TETRAGONOLOBA* AND THEIR BIOEFFICACY

For registration to the degree of

Doctor of Philosophy

IN THE FACULTY OF SCIENCE

THE IIS UNIVERSITY, JAIPUR

Submitted by

Priya Kumari Jain

ICG/2013/16410

Under the supervision of

Dr. Shilpi Rijhwani

Department of Botany

May 2014

Table of contents

S.No.	Content	Pg. no.
1.	Introduction	3-8
2.	Review of literature	9-16
3.	Objectives of study	17
4.	Significance of study	18
5.	Methodology	19-21
6.	Plan of work	22
7.	References	23-24

INTRODUCTION

The use of local plants in folk medical practices has a long history. The resource base of the traditional medical practices prevalent in rural and tribal villages of India and abroad is mainly the plants. (Lakshman *et al* 2012) The plants which have medicinal properties are used to maintain and promote healthy life, prevent disease and cure ailments. Record show that even today, 80% of the world population depends on herbal traditional medicine for their primary health care. (Qureshi *et al* 2008).

Plants are rich in a wide variety of secondary metabolites like tannins, terpenoids, alkaloids and flavanoids, which have been found antimicrobial properties. Laboratories are all over the world have found, literally, thousands of phytochemicals which have inhibitory effects on all types of micro-organisms (Cown 1999).

The meaning of phytochemistry is the study of phytochemicals. These are chemicals derived from plants. Phytochemicals are non- nutritive plant chemicals that have protective or disease preventive properties. They are nonessential nutrients, means they are not required by the human body for sustaining life. We known that plant produces these chemicals to protect them-selves but recent research demonstrate that they can also protect humans against diseases. (Lakshman *et al* 2012).

In the field of phytochemistry, the techniques which are commonly used are extraction, isolation and structural elucidation (MS, 1D and 2D NMR) of natural products, as well as various chromatography techniques (HPLC, GC-MS). Phytochemistry is mostly used in the field of indigenous medicine especially in the field of herbal medicine.

For thousands of years, medicine depended exclusively on leaves, flowers and barks of plants because they consist of a wide variety of chemical compounds that offer a promising source of new antimicrobial agents. The use and search for drugs and food supplements obtained from natural sources like plant extracts have increased in recent years. Plant are rich source of secondary metabolites are which exhibit significant pharmacological effects. Recently many new synthetic drugs have come into use and in many instances these are carbon copies of chemicals identified in plants (Conway 1973). Hence phytochemical methods are important to screen and analyze bioactive components, not only for the quality control of crude drugs but also for the elucidation of their therapeutic mechanism.

The Fabaceae or Leguminosae commonly known as the legume, pea, or bean family, are a large and economically important family of flowering plants. It includes trees, shrubs and herbaceous plants perennials or annuals, which are easily recognized by their fruit (legume) and their compound, stipulated leaves. Records demonstrate that this group is widely distributed and is the third-largest land plant family in terms of number of species, behind only the Orchidaceae and Asteraceae, with 730 genera and over 19,400 species. The largest genera are Astragalus (over 2,400 species), Acacia (over 950 species), Indigofera (around 700 species), Crotalaria (around 700 species),

and Mimosa (around 500 species), which contain around 9.4% of all flowering plant species. Tropical rainforests and dry forests in America and Africa are where the Fabacea family is most commonly found .

Many member of Fabaceae family show a special character. Their roots are colonized by a bacteria known as Rhizobium that have the ability to take nitrogen gas (N_2) out of the air and convert it in the form of usable nitrogen in the form of NO_3^- or NH_3 . This process is called nitrogen fixation. In this relationship, the legume, acting as a host, and rhizobia, acting as a provider of usable nitrate, form a symbiotic relationship. In all Fabaceae family comprises of 470 genera and 14,000 species.

Cyamopsis tetragonoloba which is commonly known as Guar has been grown in India since ancient time for vegetables and fodder purposes. At present it is grown more for mucilaginous gum production. The presence of a number of wild relatives of guar in Africa suggests that it was most probably originated in Africa. In India, it is grown in Rajasthan, Gujarat, Haryana, Punjab, Uttar Pradesh, Madhya Pradesh and Orissa. Guar plant produces a cluster of flowers, therefore, it is also known as cluster bean. Its taproot system is well developed. Therefore, it is also popular as drought hardy plant and can survive better under comparatively dry conditions.

Classification (as per Bentham and Hooker system) :

Kingdom: Plantae

Division: Dicotyledonae

Order: Rosales

Family: Fabaceae

Sub family: Papillionatae

Genus: *Cyamopsis*

Species: *tetragonoloba*

Guar is a coarse, upright, bushy, drought-resistant, shrub ranging from2 to 9 feet in height. It has pointed , saw-toothed, trifoliate leaves up to 10 cm long and small purplish flowers borne along the axis of a spikelet. The pods are straight, hairy, pale green, up to 12 cm long and contain 5 to 12 hard seeds (beans) each. There are both dwarf and tall cultivars. Guar flowers are self- pollinating. A mature unopened bud starts out white and then changes to a light pink as petals begin to open. Finally, the flower is deep blue. However, the plant morphology is highly variable. Guar has a deep tap root system and with the help of this they can find moisture deep below the soil surface. (Lakshman *et al* 2012)

C. tetragonoloba L. is a well-known traditional plant used in folklore medicine. It acts as an appetizer, cooling agent, digestive aid, laxative, and is useful in dyspepsia and anorexia and has anti-ulcer, anti-secretory, cytoprotective,

hypoglycemic, hypolipidemic and anticancer effects. (Mukhtar *et al* 2004) The pods contains condensed tannins together with p-coumaric, caffeic, gallic and gentisic acids, quercetin, kaemfeol and its 3-arabinoside, p-hydroxycinamyl and coniferyl and coniferyl alcohol.

Because of its nutritional qualities, beans can successfully replace meat. Its rich containment of proteins (approximately 70% of the necessary amino acids which can be found in meat), carbohydrates and the lack of toxins, it is best recommended for its beneficial effects on health. Green beans contain about twice as much iron than spinach. Besides its protein content, this bean also contains organic and mineral substances (sodium, potassium, calcium, magnesium, silica, nickel, copper, and cobalt), vitamins (A1, B1, B2, B5, B6, C, E, K and P1). Green bean carotenoids include lutein, beta- carotene, violaxanthin, and neoxanthin and flavonoids include quercetin, kaemferol, catechins, epicatechins, and procyanidins. Bean also contains fibers (soluble and insoluble) with an important role in the body: it reduces the level of cholesterol, prevents constipation and colon cancer. Beans also benefit the immune system through its anti- infectious effect which facilitates the growing of the number of leucocytes, destroying injurious microorganisms in the body. This effect is present because of chromium - a substance present in beans with the role of stimulating lymphatic ganglions . (Chaurasia *et al* 2012).

The Present studies deals with the identification of phytochemicals and there quantitative estimation, antimicrobial activity of *Cyamopsis tetragonoloba* and its important medicinal qualities.

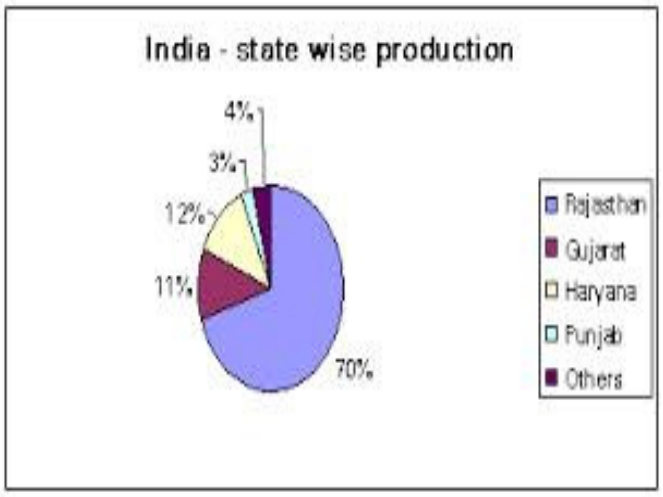


Fig: showing production of *Cyamopsis tetragonoloba* in India

Fig: A twig of *Cyamopsis tetragonoloba* (Guar)

Fig: Seeds of *Cyamopsis tetragonoloba*

REVIEW OF LITERATURE

Khan *et al* (1981) estimated the effect of guar gum in capsule form on serum total cholesterol, triglycerides, and lipoprotein cholesterol (very low-density lipoproteins, low-density lipoproteins, and high-density lipoproteins) in healthy volunteers in a double-blind study.

Sarathy *et al* (1983) shows that tender cluster bean pod (*Cyamopsis tetragonoloba*) is a vegetable commonly consumed in South India and contains 20% of guar gum on dry weight basis. Effects of incorporating this vegetable into a hyper cholesterolemic diet of rats were studied. All test materials--cluster bean 0.5 and 2.5% levels, commercial guar gum (0.5%), cluster beans partially free from guar gum (1.6%), neutral detergent fiber of cluster bean (0.6%)--exerted a hypo cholesterolemic effect and also lowered liver cholesterol concentration, but did not alter liver weights. Neutral detergent fiber of cluster bean and guar gum lowered cholesterol levels.

Khatta *et al* (1988) examined four cultivars of *C. tetragonoloba* L. and found 24.5-32.9% crude proteins, 2.4 - 3.3% crude fats, 3.2 - 4.0% ash and 9.0 - 10.2% crude fibers. Hafedh *et al* (1998) reported that the *C. tetragonoloba* L. beans had 32.81% crude proteins, 3.18% crude fats, 4.19% ash and 10.87% crude fibers.

Miettinen *et al* (1989) examined short-term viscous dietary fibres, Plantago ovata and guar gum preparations, decreased serum cholesterol, mainly LDL cholesterol, as compared to low fibre or nonviscous high fibre periods, through enhancing cholesterol elimination as fecal bile acids. These changes were associated with significant increases in serum levels of cholesterol precursors, both in methylsterols and demethylated precursor sterols, while that of cholestanol (saturated cholesterol derivative) was decreased.

According to Todd *et al* (1990) guar gum has pharmacological properties and can be used as a dietary adjunct in hypercholesterolaemia. Clinical trials indicate that, when used alone, guar gum may reduce serum total cholesterol by 10 to 15%. Guar gum as monotherapy may be considered at most modestly effective in reducing serum cholesterol levels.

Oral feeding of the aqueous extract of beans of *Cyamopsis tetragonoloba* was investigated on fasting blood glucose levels in glucose loaded, normal and alloxan-induced diabetic rats and compared with gliclazide, a reference drug. The aqueous extract of beans at 250 mg/kg body wt significantly lowered blood glucose levels in alloxan-induced diabetic rats within 3 hr of administration. Continued administration of the extract at the same dose daily for 10 days produced statistically significant reduction in the blood glucose levels of alloxan induced diabetic rats while marginal activity was seen in normal and glucose-loaded rats. (Mukhtar *et al* 2004)

Brennan (2005) explain the use of dietary fibres, especially soluble dietary fibres (such as guar gum, locust bean gum, and *Psyllium* fibres), resistant starch, and slowly digestible carbohydrates. These have been shown to alter food structure, texture, and viscosity, and hence the rate of starch degradation during digestion. And shows association between the rate of carbohydrate degradation during digestion, and the regulation of postprandial blood sugar and insulin levels.

Kaushal *et al* (2006) outlined that guar beans contained flavonoids in the range of 0.13 to 0.23%. And also reported that gallic acid as well as its derivatives, kaempferol, caffeic acid and ellagic acid were derived from *C.tetragonoloba* L. beans.

Mukhtar *et al* (2006) shows that *C. tetragonoloba* L. is a well-known traditional plant used in folklore medicine. It acts as an appetizer, cooling agent, digestive aid, laxative, and is useful in dyspepsia and anorexia , anti-ulcer, anti-secretory, cytoprotective, hypoglycemic, hypolipidemic and anti-hyperglycemic effects.

Kays *et al* (2006) found that the protein, fat, carbohydrate and ash contents of *C.tetragonoloba* L. beans were ranged between 22.9 - 30.6%, 2.88 - 3.45%, 50.2 - 59.9% and 3.04 - 3.53%, respectively.

Prieto *et al* (2006) shows Short-term and long-term effects of guar on postprandial plasma glucose, insulin and glucagon-like peptide 1 concentration in healthy rats. And examined guar gum decreases postprandial glycemia and insulinemia and improves sensitivity to insulin in diabetic patients and several animal models of diabetes.

According to Ozaslan *et al* (2007) there is a growing trend in the use of medicinal plants because of their medical effectiveness and low toxicity. As a results many natural anticancer agents have been derived from these.

Guar beans are potentially high sources of additional phytochemicals. (Wang *et al* 2007) He stated that *C. tetragonoloba* L. beans are a good source of quercetin and kaempferol.

Cyamopsis tetragonoloba (L) Taub is a moderate sized annual herb found throughout India as a cultivar for its pods used as vegetable. Plant is popular in indigenous system of medicines like Ayurveda, Siddha and discussed in various traditional literatures. In traditional medicines various parts such as leaves, seeds and pods are used in diabetes, asthma, inflammation, as laxative, antibilious agents, appetite depressor (weight loss not observed) and hypolipidemic agent. (Sharma *et al* 2007)

Therapeutic and medicinal properties lies in the soluble dietary fiber content of guar gum to improve the serum biochemical profile of human and non-human primates, reducing total serum cholesterol, triglycerides, increasing the high density lipoprotein cholesterol level, and the management of glycemic indices and obesity and it is effective in reducing the cardiovascular disease risk, diabetes and weight loss programs. (Butt *et al* 2007)

According to Goyali *et al* (2007) fleshy green tender pods of cluster bean, are a typical tropical warm climate vegetable crop, grown in subtropical area during summer and used as vegetable. The rough shined hairy types are used as fodder for the cattle and as green manuring crops. It is rich source of protein (3.2%), fibre (3.2%), carbohydrate (10.8%), calcium (130 mg%), phosphorus (57 mg%), and iron (0.6 mg%). Galactomanan, the essential ingredient of guar gum is highly mucilagnous and used in various industries. Guar gum of cluster bean being rich in soluble fibre, i.e. non starchy polysaccharides is useful for patients suffering from obesity, diabetes and hyperlipidemia.

Hypocholesterolemic effects associated with soluble fiber consumption are clear from animal model and human clinical investigations. Guar gum use as soluble fibers in the regulation of cholesterol metabolism. (Rideout *et al* 2008)

Hassan *et al* (2009) reported haemolytic and antimicrobial activities of saponin-rich extracts from guar meal. Results indicated that only 100% MeOH fraction and its 16 min peak sub-fraction exhibited both haemolytic and anti- bacterial activities against *Staphylococcus aureus, Salmonella typhimurium* and *Escherichia coli,* but 20% and 60% MeOH fractions stimulated *Lactobacillus* spp. growth.

According to Sharma *et al* (2010) *Cyamopsis tetragonoloba* is a weed of Rajasthan and has ethno-botanical importance. When land is cultivated to raise crops, weeds

spring up naturally along with the crop plants. Weeds are defined as "a plant out of place or an unwanted plant or a plant with a negative effect or plants that compete with man for the soil" (Kasera *et al* 1998).

Plants that are specifically employed for the treatment of diabetes are *Acacia nilotica, Acacia senegal, Aegle marmelos, Calotropis procera, Capparis deciduas, Cassia auriculata, Cassia sophera, Cayratia trifolia, Cyamopsis tetragonoloba, Dalbergia sisso, Gymnema sylvestre, Momordica charantia, Syzygium cumini, Withania somnifera.* (Batra *et al* 2011)

Thirty one genotypes of clusterbean [*Cyamopsis tetragonoloba (L.)*] were evaluated to estimate variability, broad sense heritability and genetic advance over mean for vegetable pod yield and related attributes, during Kharif 2009. Maximum range of variability was observed for number of branches, plant height, clusters per plant, pod length and pod yield per plant. High heritability coupled with high genetic gain in percentage was observed for pod yield per plant, number of pods per plant, days to 50% flowering, number of branches and plant height. High heritability with low genetic advance was shown for pods per cluster, number of seeds per pod and pod width. The results of present investigation suggests that selection based on plant height, number of branches, days to 50% flowering, clusters per plant and pod yield per plant might bring simultaneous improvement in vegetable pod yield of cluster bean.(Shabharshrai *et al* 2011).

Mohamed *et al* (2011) observed biochemical studies on *Plantago major* L. and *Cyamopsis tetragonoloba* L. Guar beans had high contents of proteins, fats and total hydrolysable carbohydrates. *Plantago* leaves had high percentage of linolenic acid (56.19%), While its seeds and Guar beans had high percentages of linoleic acid (25.41 and 48.99%, respectively). Essential and non essential amino acids

were present in all samples and Guar beans had high amounts of glutamic, arginine, aspartic and leucine.

Cyamopsis tetragonoloba fruit and *Cyperus rotundus* rhizome were analysed by GC-MS analysis to assess their phytochemical constituents. The ethanolic extract of *C. tetragonoloba* fruit showed the presence of thirty four phytochemical constituents. The major phytochemical compounds were inositol, ethyl alpha-d-glucopyranosid end stigmasterol. The ethanolic extract of *C. rotundus* showed the presence of twenty two phytochemical constituents. The major phytochemical compounds were 7-isopropenyl-1,4a-5,6,7,8-hexahydro-3H-naphthalen-2-one, zierone and (+)-cis-longipinan. (Surendran *et al* 2012)

Quality assessment of guar gum (Endosperm) of guar (*Cyamopsis tetragonoloba*) was done by Murwan *et al* (2012) Six guar genotypes were used for this study The results revealed chemical compositions of endosperm of the guar seed as : 4.8-8.7 % moisture, 3.5-5.5 % protein, 0.5-0.9 % fat, 0.5- 1.3 % ash, 1.4- 2.0 .% fiber and 83.3-87.5 % carbohydrates.

Pande *et al* (2012) shows that Cluster beans (*Cyamopsis tetragonoloba*) are rich source of soluble fiber content and are known for their cholesterol lowering effect. The beneficial anti-hyper cholesterolaemic effect of whole dietary cluster beans as a source of dietary fiber was evaluated in high cholesterol diet induced hyper cholesterolaemia in experimental rats.

Chaurasia *et al* (2012) shows the antibacterial activity of four different varieties of green beans. Fresh extracts of pods of *Phaseolus vulgaris* (french beans), *Vicia faba* (broad beans; saem), *Cyamopsis tetragonoloba* (cluster beans; guar) and *Vigna unguiculata* (cowpea; lobia) were tested against two human pathogenic bacteria: *Escherichia coli* (gram negative) and *Bacillus subtilis* (gram positive)

using disc diffusion assay. The streptomycin was used as the control. The results showed that all the extracts possess antibacterial activity (bacteriostatic activity). The range varied from 9 mm to 15 mm against *E.coli* and 10 to 12 mm against *B. subtilis*.

Sharma *et al* (2012) analysed genetic and Phytochemical constitute of Cluster bean (*Cyamopsis tetragonaloba* L.) by RAPD and HPLC in which five varieties of guar RGC-936, 1002, 1003, 1031 and 1017 were taken. Phenolic acids such as sinapic acid, cholorogenic acid, caffeic acid and gallic acids were detected among all cultivars. Whereas flavanoids i.e. kaempferol and myricetin were showing variations among all cultivars. The phytochemical analysis of guar may expand its nutraceutical and pharmaceutical utilization and information from this study will be useful to breeding programmes for improving guar seed quality .

Lakshman *et al* (2012) done the phytochemical screening, quantitative estimation total phenolics and total flavonoids, anti microbial evaluation of *Cyamopsis tetragonoloba*. According to them Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. They are nonessential nutrients, meaning that they are not required by the human body for sustaining life. It is well-known that plant produces these chemicals to protect themselves but recent research demonstrate that they can also protect humans against diseases. *Cyamopsis tetragonoloba* is also traditionally known used as a laxative. It is also used for treating diarrhea, irritable bowel syndrome (IBS), obesity, and diabetes, for reducing cholesterol. The medicinal properties of the plant are due to the anti oxidants present in the plant. Present studies deals with the identification of phytochemicals and there quantitative estimation, antimicrobial activity of *Cyamopsis tetragonoloba* and its important medicinal qualities provided

one does not play around with poisonous plants they are totally free of harmful side effects - unlike the modern drug industry.

Singh *et al* (2013) Show that ethanol and aqueous extracts from leaf, stem and fruits of *Cyamposis tetragonoloba* (L.) Taub. were investigated for their anthelmintic activity by using four concentrations (12.5, 25, 50, 100 mg/ml) of each extract against *Pheretima posthuma* as test worms.

According to Srinivasan *et al* (2013) Soluble fiber-rich tender cluster bean pod (*Cyamopsis tetragonoloba*) and a combination of CB and capsaicin, a thermogenic spice compound, were evaluated for weight-reducing effect in high-fat-fed Wistar rats.

According to Pandey *et al* (2013) Gastro intestinal protective effect of soluble, dietary fiber-rich tender cluster beans, with respect to the activities of antioxidant enzymes and the concentration of antioxidant molecules, was examined in Wistar rats. Dietary intervention with tender cluster beans significantly enhanced the activities of antioxidant enzymes and the concentrations of antioxidant molecules in both gastric and intestinal mucosa. The gastroprotective effect of the cluster bean was also reflected in its positive effect on gastric mucosal glycoproteins, resulting in a lowering of mucosal injury. Incidentally, the serum and liver also showed an elevated antioxidant status, thus suggesting desirable lowered oxidative stress results when tender cluster beans are consumed.

OBJECTIVES

Considering the above facts, this research work entitled, "Comparative phytochemical studies of two varieties of *Cyamopsis tetragonoloba* and their bioefficacy" has been taken up with the following objectives:

1. To collect healthy plant seeds and confirm their variety .
2. To analyze physicochemical constituents of the plant.
3. To purify and identify the isolated component by chromatographic techniques.
4. To study the biological activity of the crude extracts as well as the components. (antibacteria or antihelminthic activity)
5. To elucidate the structure of the isolated components by spectroscopy.
6. Identify the hypolipidemic and hypocholesterolemic effect of plant extract as well as the component on particular sample.

SIGNIFICANCE

It is widely accepted that research on medicinal plants should be focussed primarily on species whose pharmaceutical activities have already been demonstrated. The present study is therefore designed to provide scientific evidence for the selected plants to be used as a traditional folk remedy by investigating the phytochemical constitution of the plant as well as the antibacterial activity. Essential oils would be evaluated for antimicrobial activity against gram-positive and gram- negative bacteria strains obtained from standard agencies. Developing countries have the potential of plant resources that exhibit a wide range of biological activities which may help in the development of cheaper and more potent anti-microbial agents. Many plants have been used because of their antimicrobial traits, which are chiefly synthesized during secondary metabolism of the plant. Nearly, all culture and civilizations from ancient times to the present day have depended fully or partially on herbal medicine because of their effectiveness, affordability, availability, low toxicity and acceptability. Therefore, newer plants should be investigated to better understand their properties, safety and efficacy.

The abuse of drugs for ailments is increasing with time which leads to side effects & sometimes death. It is, therefore, very important to search for effective but low cost and reliable traditional therapeutic agents. This work is therefore aimed at studying of photochemical properties of two varieties of *Cyamopsis tetragonoloba* and their biological activities.

METHODOLOGY

1. Identification and authentication of plant seeds of *Cyamopsis tetragonoloba* .

2. Samples which are collected are shade dried and transported to the laboratory in accordance with standard procedures.

3. Seeds will be selected as a sample from *Cyamopsis tetragonoloba*.

4. Powdering of shade dried Plant material for extraction.

5. Hot sohxlet extraction for various phytohemicals using solvent series (n-hexane, chloroform, ethyl acetate, methanol and water) followed by Physico-chemical analysis test according to Harborne's standard guide.

6. Qualitative estimation

- Tests for carbohydrates

  (By Molisch's test, Fehling's test, Benedict's test)

- Tests for glycosides

  (By Legal's test, Borntrager's test)

- Tests for alkaloids

  (By Mayer's test, Dragendroff's test, Hager's test, Wagner's test)

- Tests for Phyto steroids

  (By Salkowski test, Liebermann- Burchard test)

- Tests for flavanoids

(By Shinoda test, Ferric chloride test, Alkaline reagent test)

- Tests for saponins

  (By foam test)

- Tests for Proteins and amino acids

  (By Biuret test, Million's test, Xanthoprotein test, Ninhydrin test)

- Tests for Tannins and Phenolic compounds

  (By 5 % $Fecl_3$ sol., Lead acetate sol., Acetic acid sol., Dilute iodine sol., Dilute HNO_3)

- Tests for fixed oils and fats

  (By Spot test, Saponification test)

7. Quantitative estimation -

(Estimated content is calculated as % of the starting materials)

8. Extracted crude samples will be redissolve in respective solvents & preserved as mother sample for further analysis of biological activity.

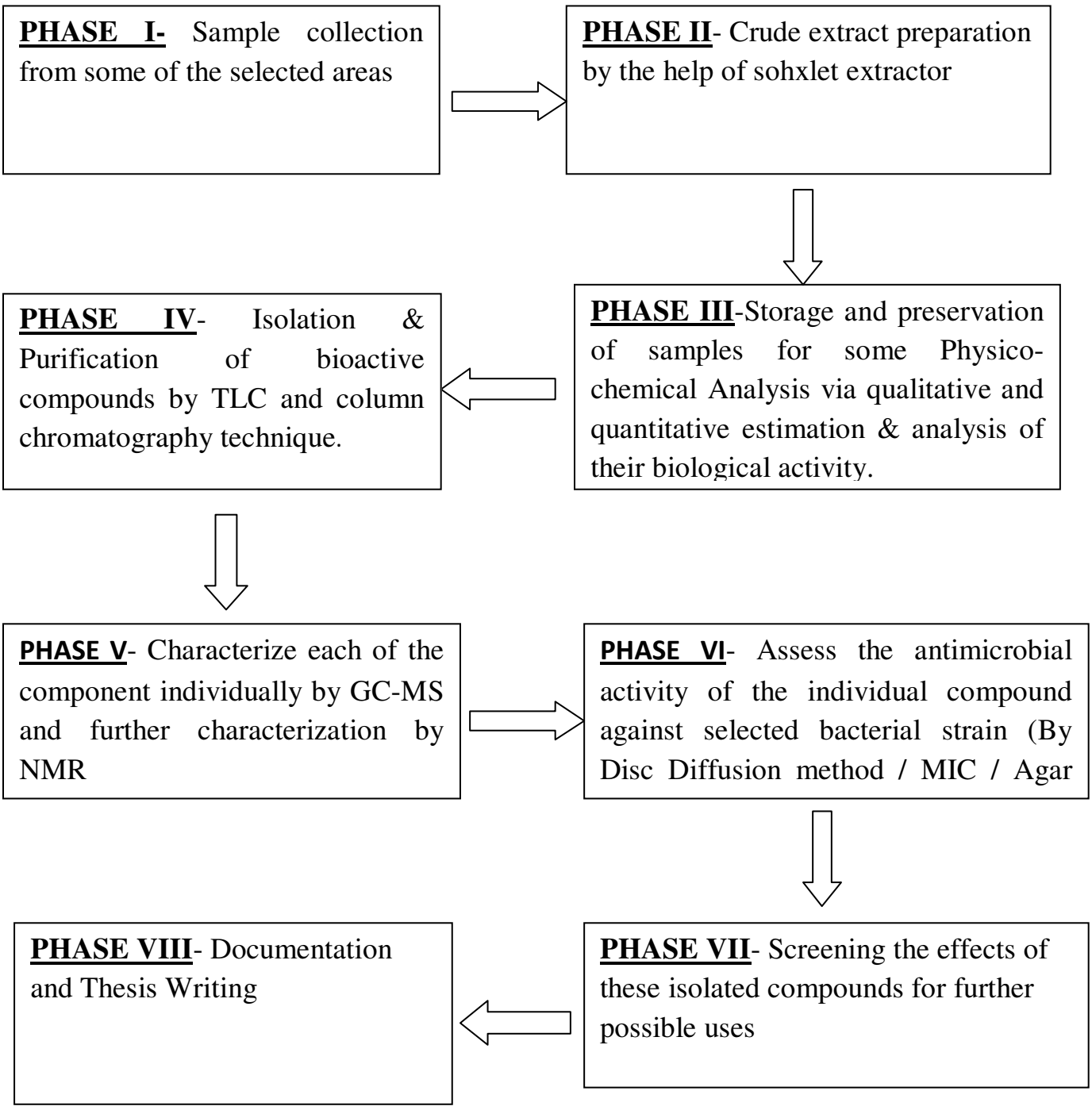
9. Isolation -

Samples collected will be processed further for Isolation, Purification and Identification of unknown compounds using Chromatography technique. (Thin layer chromatography or Column Chromatography)

Characterization each of the component individually by some standard techniques by GC-MS & NMR.

10. Purified extract will be treated against selected bacterial strains (by Disc Diffusion method / MIC / Agar well Diffusion method or Kirby-Bour method 1961) for further assessment of antimicrobial activity & evolution of the various biochemical parameters.

PLAN OF WORK


PHASE I- Sample collection from some of the selected areas
PHASE II- Crude extract preparation by the help of sohxlet extractor
PHASE III-Storage and preservation of samples for some Physico-chemical Analysis via qualitative and quantitative estimation & analysis of their biological activity.
PHASE IV- Isolation & Purification of bioactive compounds by TLC and column chromatography technique.
PHASE V- Characterize each of the component individually by GC-MS and further characterization by NMR
PHASE VI- Assess the antimicrobial activity of the individual compound against selected bacterial strain (By Disc Diffusion method / MIC / Agar
PHASE VII- Screening the effects of these isolated compounds for further possible uses
PHASE VIII- Documentation and Thesis Writing

REFERENCES

- Khan, AR., Khan, GY., Metchel, A. and Qadeer, MA. (1981): Effect of guar gum on blood lipid. *Am. J. Clin. Nutr*. 34: 2446-2449.

- Sarathy, R. and Saraswati, G. (1983): Effect of tender cluster bean pods on cholesterol levels in rats. *Am.J. Cli.Nutr*. 38: 295-9.

- Khatta, VK., Kumar, N. and Gupta, PC. (1988): Chemical composition and amino acid profile of four varieties of guar (*Cyamopsis tetragonoloba*) seed. *Indian J. Anim. Nutr.* 5 : 326-326.

- Miettinen, TA. and Tarpila, S. (1989): Serum lipids and cholesterol metabolism during guar gum, *plantago ovata* and high fibre treatments. *Clin. Chem. Act*. 183: 253-62.

- Todd, PA., Benfield, P. and Goa, KL. (1990): Guar gum-A review of its pharmacological properties, and use as a dietary adjunct in hypercholesterolaemia. *Drugs.* 39: 917-28.

- Cown, M. M. (1999) : Plant products as antimicrobial agents. *J. Clin. Microbiol.* 12: 564-582.

- Mukhtar, H.M., Ansari, S.H., Ali, M., Bhat, Z.A. and Naved, T. (2004): Effect of aqueous extract of *Cyamopsis tetragonoloba* Linn. beans on blood glucose level in normal and alloxan- induced diabetic rats. *Indi. J. Experi. Bio*. 42: 1212-1215.

- Prieto, PG., Cancelas, J., Villanueva-Penacarrillo, ML., Malisse, WJ. and Valverde, I. (2006): Short-term and long-term effects of guar on postprandial plasma glucose, insulin and glucagon-like peptide 1 concentration in healthy rats. *Hame Meta. Rea.* 38: 397-404.

- Ozaslan, M., Karagöz, K., Kalender, ME., Kilic, IH., Sari, I. and Karagöz, A. (2007): In vivo Antitumoral effect of *Plantago major* L.extract on Balb/C Mouse with Ehrlich Ascites Tumor. *Am. J. Chin. Med.* 35: 841- 851.

- Butt, MS., Shahjadi, N., Sharif, MK. and Nasir, M. (2007): Guar gum a miracle therapy for hypercholesterolemia, hyperglycemia and obesity. *Crit.Rev.FoodSci. nutria*.47: 389-96.

- Wang, ML. and Morris, JB. (2007): Flavonoid content in seeds of guar germplasm using HPLC. *Plt. Gen. Res. Char. Uti.* 5: 96-99.

- Rideout, TC ., Harding, SV., Jones, PJ. and Fan, MJ. (2008): Guar gum and similar soluble fibers in the regulation of cholesterol metabolism: current understandings and future research priorities. *Vasc. Health Risk Manage* .4: 1023-1033.

- Hassan, SM., Haq, AU., Byrd, JA., Berhow, MA., Cartwright, AL. and Bailey, CA. (2009): Haemolytic and antimicrobial activity of saponin- rich extract from guar meal. *Food chem.* 119: 600-605.

- Sharma, L. and Khandelwal, S. (2010): Weeds of rajasthan and their ethno botanical importance. *Ethno. Medi*. 4: 75-79.

- Mohamed, IK., Osama, MAF., Samiha, MAS. and Zahrat, EMM. (2011): Biochemical studies on *Plantago major* L. and *Cyamopsis tetragonoloba* L. *Inter. J. Biodiv.Conser*. 3: 83-91.

- Pande, S. and Shrinivasan, K. (2012): Potentiation of hypolipidemic and weight-reducing influence of dietary tender cluster bean (*Cyamopsis tetragonoloba*) when combined with capsaicin in high-fat-fed rats. *J. Agri. Food Chemi*. 60: 855-62.

- Chuarasia, S. and Saxena, R. (2012): Antimicrobial activity of four different varieties of green beans. *Res. J. Phar. Bio. Che. Scie.* 3: 70-74.

- Pande, S., Platel, K. and Srinivasan, K. (2012): Antihypercholesterolaemic influence of dietary tender cluster beans (*Cyamopsis tetragonoloba*) in cholesterol fed rats. *Indian J. Med.Res*. 135: 401-406.

- Surendran, S. and Vijayalakshmi, K. (2012): GC-MS Analysis of phytochemicals in *Cyamopsis tetragonoloba* fruit and *Cyperus rotundus* rhizome. *Inter. J. Phar. Phyto. Res.* 3: 102-106.

- Badugu, L.R. (2012): Phytochemical screening, quantitative estimation total phenolics and total flavonoids, antimicrobial evaluation of *Cyamopsis tetragonoloba*. *Inter. J. Res. Phar. Bio. Scie*. 3: 1339-1142.

- Murwan, K.S., Abdalla, A.H. and Nouri, S.H. (2012): Quality assessment of guar gum (endosperm) of guar (*Cyamopsis tetragonoloba*). *ISCA J. Bio. Scie.* 1: 67-70.

- Singh, S. and Devi, B. (2013): Anthelminthic activity of *Cyamopsis tetragonoloba* (L.) taub. *Inter. J. Phar. Res. Dev*. 5: 015-021.

-